RELATIONSHIPS BETWEEN DIETARY SUGAR, CANDIDA ALBICANS, AND GASTRIC ULCERS IN SWINE

by

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INTRODUCTION

Gastric ulcers of the esophageal area in swine have received increasing attention and have been found in ever-increasing numbers since first being specifically reported in 1951. The gastric ulcers referred to in this thesis are those in the glandless esophageal area, unless noted otherwise.

The etiology of gastric ulcers in swine remains questionable, but is of primary importance. It is difficult, at best, to diagnose the condition in the live animal under normal field conditions. Treatment is symptomatic and control is unknown. Treatment, prevention and control, and possibly diagnosis could be performed in a more efficient manner if the etiologic agent were known.

Many etiologic agents and conditions have been suggested, usually without conclusive experimental results or survey data. *Candida albicans* has been suspected as a cause of gastric ulcers in swine (Griffing, 1963; Bixby, 1964). Dietary sugar has been felt to aid growth of and/or pathogenicity of *C. albicans* in the upper digestive tract of swine (Osborne, 1960).

The experiment herein reported attempts to determine three relationships: 1. Between dietary sugar and growth of *C. albicans* in the porcine digestive tract; 2. Between dietary sugar and presence of pathologic changes in the esophageal area of the porcine stomach; and 3. Between growth of *C. albicans* and pathologic changes in the esophageal area of the porcine stomach.

Secondarily, the experiment attempts to determine if the blood glucose level has any effect on the above relationships.

REVIEW OF LITERATURE

Although occurring previously, ulceration of the esophageal area of the porcine stomach was first reported as a separate entity by Bullard (1951). In previous reports, esophagogastric ulcers were considered as part of another disease or were discussed in conjunction with ulcers located in other areas of the stomach (Kernkamp, 1945).

Kowalczyk (1960), in a series of experiments from 1957 to 1959, found stomach ulcers in swine not uncommon but incriminated no etiologic agent. He suggested that the seasonal factor might play a causal role.

In New Zealand, Dodd (1960) reported on ulcerative gastritis in swine caused by the stomach worm, Hyostrongylus rubidus. However, the fundus of the stomach was primarily involved.

A white necrotic area around the esophageal opening in the stomach of 1 of 4 pigs on a 0.4% copper sulfate diet and esophagogastric ulceration of 1 of 3 pigs on a 0.2% copper sulfate diet were reported by Allcroft (1961). However, these were the only lesions of this type reported and were associated with a marked orange discoloration of the liver. Liver change appeared in 3 other pigs which had no gross pathologic change in the esophagogastric area.

Deaths due to esophagogastric ulcers in pigs unintentionally fed high copper diets were reported by Buntain in England (1961). He concluded that some unrecognizable toxic factor in the diet in addition to the excessive copper level was responsible for the deaths. Moderate to severe liver abnormalities were noted in all cases.

Perry (1962) reported greater than 50% incidence of gastric ulcers in swine fed corn gelatinized by a pelleting machine. Nuwer (1963) reported 16 to 60% incidence of ulceration in pigs in feeding trials attempting to counteract the "ulcerogenic" effect of gelatinized corn with oxytetracycline, copper, vitamins A, E, K, and methionine. No conclusive counteraction was reported. Increased keratinization of esophagogastric epithelium was present in almost 100% of the test animals.

In Ireland, Barron and O'Brien (1963) formed the opinion that an outbreak of hemorrhage and gastric ulcers of the cardiac region of 13- to 15-week-old pigs was due to flaked concrete in the pens from which the animals were floor-fed.

Reese (1963), in seven trials involving 352 pigs, concluded that some antibiotics and some ration changes did not cause gastric ulcers; that many individual vitamins at recommended levels did not decrease stomach abnormalities; that a beneficial effect was noted from feeding a wheat-oat ration in place of a corn ration, feeding on alternate days, and feeding a wet ration twice daily; and that a detrimental effect was obtained from crowding.

Results obtained by Reese (1964) indicate that the feeding of oats as part or all of the cereal portion of the ration may be effective in preventing or retarding ulcer formation in swine.

High nitrite feeds, which might interfere with vitamin A synthesis and metabolism, and genetic factors were suggested by Rothenbacher (1963) as possible causes of esophagogastric ulcers.

Perry (1963) reported a much higher incidence of esophagogastric ulceration, cornification, and erosion in pigs fed heat-treated yellow corn as compared to ground raw yellow corn. Supplemental thiamine, vitamin B_{12}, lysine, and fish meal failed to reduce the incidence of ulceration.

Curtin (1963) reported a higher incidence of gastric ulcers in swine in late spring and early winter. Rapid growth of the pigs was suggested as a contributing factor.

In trials conducted by Mahan (1964), results indicated that reduced growth rate, presence of dietary fiber, or dried skim milk with gelatinized corn did not reduce ulcer incidence. In a trial using varying particle sizes of raw ground corn, ulcer incidence was low overall, but was markedly higher with fine particle size as compared to coarse particle size.

Nuwer (1965) reported that the ulcerogenic effects of a gelatinized corn-soybean meal ration were not prevented by the addition of oxytetracycline, oxytetracycline plus copper sulfate, 2,000 or 5,000 IU of vitamin A/lb. of feed, tocopherol, tocopherol acetate, menadione, methionine, an amylolytic-proteolytic enzyme, librium hydrochloride tranquilizer, an antihistamine, or dried

distillers' solubles. Hemoglobin and hematocrit studies did not prove to be reliable criteria for detecting chronic esophagogastric ulcers. The results also indicated that no interference of vitamin A absorption was present. Rats fed the same diets producing ulcers in swine did not develop ulcers.

Many case reports are present in the literature of enteric diseases or conditions associated with *C. albicans* which may possibly have some relationship to the esophagogastric ulcer problem in swine.

Blaxland and Markson (1954) reported moniliasis in turkey poults with *C. albicans* as the causative agent. Hyphae could remain on the surface of, and a few could even grow into, the crop epithelium without apparent reaction. But, if penetration increased, surface squames piled up as debris, while epithelial proliferation and keratosis increased below the site of reaction.

Gitter (1959) isolated *Rhizopus microsporus* from lesions in the stomachs in an outbreak of deaths in suckling pigs. *C. albicans* also was isolated from the pigs, as well as from the straw, air, and drinking water of the pen.

C. albicans was isolated from artificially reared piglets showing lesions of moniliasis (Osborne, 1960). The pathologic changes were present in the superficial layers of the mouth, esophagus, and stomach. The authors felt that devitalizing factors and/or a lactose-rich diet were exciting causes. They also thought it unlikely that *C. albicans* was of primary significance.

C. albicans was isolated from the stomach mucosa of 2- to 8-week-old pigs involved in an outbreak of diarrhea on eight farms in Wisconsin. Baker and Cadman (1963) reported plaques and ulcers in the stomach, especially the esophageal area, and in the esophagus. The authors felt that C. albicans could have been primary or secondary.

Curtin (1963) thought C. albicans, which was consistently isolated from craters of esophagogastric ulcers of swine studied, was a secondary invader due to loss of stratified squamous epithelium.

Rothenbacher (1963) described in detail the gross and microscopic pathologic changes in the squamous portion of the stomach of the pig in the cardiac ulcer-gastrorrhagia syndrome. Early and/or chronic subclinical lesions were characterized by a parakeratotic proliferative change of the squamous epithelium. Progressive necrosis, erosion, and ulceration could lead to sudden hemorrhage. Histologic changes in the Malpighian and basal layers of the squamous mucosa consisted of edema of intercellular spaces with microvesiculation, hydropic degeneration, and lack of keratinization.

The esophagogastric ulcers in 87 of 443 swine studied by Curtin (1963) were usually shallow and did not penetrate beyond the muscularis mucosa. Ulcers were classified according to presence of clinical signs and duration as peracute, subacute or chronic, and subclinical.

In a study of 594 porcine stomachs, Muggenburg (1964) reported esophagogastric ulcers as being much more common than ulcers in the glandular regions. Lesions were classified according to histopathologic appearance as epithelial changes, acute erosions, subacute ulcers, chronic ulcers, and scars. Epithelial changes involved deepening of rete pegs into the tunica propria, varying depth of epithelium, and lack of true keratinization. Acute erosions were characterized by erosion of epithelium so tips of papillae of tunica propria were exposed. Subacute ulcers extended through the mucosa, sometimes into the submucosa. Chronic ulcers were characterized by involvement of a larger area, the presence of a greater amount of connective tissue, and by often extending into the tunica muscularis. Scars showed replacement of tissue beneath the mucosa by connective tissue. The stomach epithelium regenerated to cover the defect.

The possible role of dietary sugar was suggested as a contributing factor in gastric ulcers in swine (Griffing, 1963). Ellenberger (1887) showed, by use of esophageal fistula, that the sugar content of potatoes fed to pigs increased from 0.35 to 2.00% after chewing, due to the action of salivary amylase.

Hudman (1957) reported that the activity of salivary amylase decreased from an average of 0.36 mg. of maltose equivalent/ml. of undiluted saliva in 50 lb. pigs, to 0.26 mg. at 75 lbs., to 0.10 mg. at 130 lbs., using a starch substrate containing 0.1% maltose. Arkhipovets (1962) reported that the saliva of very young and very old pigs contained little amylolytic ferment.

The keratinolytic properties of *C. albicans* in the presence of glucose was demonstrated by Kapica and Blank (1957), while Blaxland and Markson (1954) indicated that the role of *C. albicans* as a pathogen may be in stimulation of keratin formation.

It is readily seen from these selected excerpts from the literature that there are no widely accepted conclusions regarding the etiology of gastric ulcers in swine. Most authors relegate *C. albicans* to a secondary role. However, in some of the non-ulcerous enteric disorders mentioned, *C. albicans* was incriminated as the primary etiologic agent. This, plus the fact that the organism has been commonly found in gastric ulcers, warrants further investigation into the role of *C. albicans*.

MATERIALS AND METHODS

Thirty pigs from four litters born within three weeks of each other at the Kansas State University Veterinary Research Laboratory were used in this experiment. All were vaccinated against hog cholera with modified live virus vaccine[1] and anti hog cholera serum[2] and against erysipelas with an avirulent oral vaccine.[3] The male pigs were castrated at about 9 weeks.

From weaning until going on separate diets, all pigs were housed in the same pen and outside area. At 18 weeks, all pigs

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[1]Alocine, Haver-Lockhart Laboratories, P.O. Box 676, Kansas City, Mo.

[2]Hog Cholera Antibody-Concentrate, Haver-Lockhart.

[3]Hydrovac, Corvel, Inc., Omaha, Neb.

were placed on the control diet. At 19 weeks, the start of the experiment, the pigs were randomly selected by numbers corresponding to ear tattoos to be placed in three lots of 10 pigs each. The three lots were put in separate pens with no outside area and were put on different diets. The distribution by lot and litter obtained by random selection is shown in Table 1.

Table 1—Random Selection Results

	Lot C	Lot S	Lot M	Total
Litter 1	2	2	2	6
Litter 2	2	2	1	5
Litter 3	2	3	4	9
Litter 4	4	3	3	10
Total	10	10	10	30

The average weights and weight ranges of the lots two weeks prior to starting the experiment were as follows: Lot C, 109.3 lbs. (98 to 130); Lot S, 110.5 lbs. (92 to 140); and Lot M, 105.0 lbs. (92 to 133). Lot C contained 7 males; Lot S, 6 males; and Lot M, 3 males.

The control lot (Lot C) was fed a normal pelletized growing ration, V36, prepared at the University feed mill. The ration contained 81.8% ground yellow corn, 15.4% soybean meal, and 2.8% vitamin, mineral, and antibiotic supplements. Lot M was fed the same ration with 2.0% technical grade maltose[1] added. Lot S was

[1]M-74, Fisher Scientific Company, Fair Lawn, N. J.

fed the control ration with 2.0% sucrose added as table sugar.

The pigs were fed free choice in self-feeders until the seventh week of the experiment. From then until the tenth week, they were limited to 7.5 lbs. of feed/pig/day on a pen basis. For the last 10 days of the experiment, all pigs were fed 10 lbs./pig/day of the control ration on a pen basis.

The lots were rotated weekly between the three pens to minimize any possible pen effect, even though the pens were the same size and in the same building.

The three lots were dosed at the same rate with live *C. albicans* (Table 2).

Table 2—*C. albicans* Dosage Schedule

Day	Means	Dosage
0	Individual oral dosage	500 million
7	Individual oral dosage	500 million
14	Individual oral dosage	500 million
17	Individual oral dosage	500 million
21	Individual oral dosage	150 million
24	Individual oral dosage	500 million
28	Individual oral dosage	800 million
32 through 48	Continuous presence in drinking water	100/ml. to 260,000/ml.
49 through 82	Water essentially free of *C. albicans*	0 to less than 50/ml.

C. albicans for oral dosage was grown on mycobiotic agar[1] and harvested at 48 hours with physiological saline solution. Cell counts were routinely performed on the saline suspension with a Coulter electronic particle counter.[2] As a check, several samples were compared with a live cell count by serial dilutions in mycobiotic agar. Very close correlation was obtained.

Pathogenicity of the organism was tested by intravenous inoculation into mice and rabbits.

C. albicans for addition to drinking water was grown in Sabouraud dextrose broth[1] for 24 to 48 hours. The drinking water counts were serial dilutions of water samples in mycobiotic agar.

Fecal samples were collected rectally from each pig by insertion and manipulation of a sterile cotton-tipped swab. Defecation could ordinarily be elicited in this manner. The samples were collected in sterile petri dishes. Samples were collected weekly from two weeks prior to ten weeks after the start of the experiment.

From each sample, 1.0 Gm. of feces was aseptically weighed and added to 49 ml. of sterile saline solution. The suspension remained for three to four hours at room temperature and was then mixed by shaking the bottle 25 times in a one-foot arc. One ml. and 0.1 ml. were then pipetted to sterile petri dishes and Pagano Levine agar[1] with added inhibitors was added. The added

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[1] Difco Laboratories, Detroit, Mich.

[2] Coulter Electronics, Inc., Hialeah, Fla.

inhibitors were cycloheximide,[1] 0.5 mg./ml., and chloramphenicol,[2] 0.05 mg./ml. These were added because the neomycin in the medium did not retard contaminant growth enough in the heavily contaminated fecal samples. The plates were mixed thoroughly, allowed to harden, and incubated at 37° C for 60 hours.

Colonies typical of _C. albicans_ (Pagano, 1957-58) were counted. Representative colonies from each plate were transferred to cuts in chlamydospore agar.[3] Sterile coverslips were placed on the cuts. The cuts were sealed at each end of the coverslip by melting a small amount of the agar with flamed sterile forceps. The chlamydospore agar was examined at two, four, and seven days for the presence of pseudohyphae and chlamydospores characteristic of _C. albicans_ (Nickerson and Mankowski, 1953).

Weekly blood glucose determinations were obtained by venipuncture of the ear and applying Dextrostix reagent strips[4] to absorb the drop of blood. The blood glucose was then obtained by comparing the resultant color to a color standard provided. These determinations were performed from two weeks prior to ten weeks after the start of the experiment.

After 81 days on the experiment, the pigs were slaughtered in a commercial abattoir. The stomachs were identified and

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[1]Acti-dione, The Upjohn Company, Kalamazoo, Mich.

[2]Chloromycetin, Parke, Davis, and Co., Detroit, Mich.

[3]Difco Laboratories, Detroit, Mich.

[4]Ames Company, Inc., Elkhart, Ind.

collected. After slitting the greater curvature and emptying most of the contents, two samples of the contents in the esophageal area were obtained with sterile cotton-tipped swabs. The contents were placed in tubes of Sabouraud dextrose broth[1] with the same added inhibitors as mentioned for the Pagano Levine agar. After thoroughly rinsing the esophageal area with potable tap water, two more swabs were taken directly from the mucosa and placed in Sabouraud dextrose broth with added inhibitors. These tubes were held at room temperature for six hours, refrigerated for 15 hours, then agitated and the swabs streaked on Pagano Levine agar with added inhibitors. These plates were incubated in pairs, one at 37° C and the other at room temperature for four days prior to the first reading. After the second reading at six days, colonies typical of _C. albicans_ were cut into chlamydospore agar as previously described. The chlamydospore agar was incubated at room temperature in a candle jar, as was done for all chlamydospore agar plates, for seven days. The plates were examined at two, four, and seven days for the presence of _C. albicans_.

The mucosa of each stomach was examined for gross pathologic change within three hours of slaughter. Special attention was given to the rectilinear, non-glandular esophageal area. Gross keratinization of the esophageal area was recorded using the scale employed by Bixby (1964). Ulceration, erosion, and other grossly visible pathologic changes were recorded.

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[1]Difco Laboratories, Detroit, Mich.

A section of tissue approximately 1 cm. by 4 cm. was taken from across the longitudinally ridged mucosa about 3 cm. from the esophageal opening toward the pylorus. These sections were aseptically placed in sterile plastic bags and frozen for several hours. They were then thawed, removed from the bags, and four pieces from each section were rubbed on the surface of and implanted sub-surface in mycobiotic agar slants. Each of the pieces of stomach contained mucosa. Duplicate tubes were incubated at 37° C and at room temperature. The tubes were read at 4, 6, 9, 14, and 21 days. At each reading, growth resembling _C. albicans_ was cut into chlamydospore agar and read at two, four, and seven days.

In addition to the above culturing, after refreezing the tissue from 8 to 12 days, each section was rethawed, macerated with a sterile mortar and pestle, and the slurry obtained inoculated on two mycobiotic slants and into two tubes of Sabouraud dextrose broth with added inhibitors. The slants and one broth tube were incubated at 37° C. The other broth tube was incubated at room temperature. In 24 hours, the broth cultures were transferred to the surface of Pagano Levine agar with added inhibitors. At this time, a Gram stain was performed to detect yeast cells. The Pagano Levine plates were incubated 72 hours at 37° C. Candida-like colonies were cut unto chlamydospore agar, which was read at two, four, and seven days.

Another tissue section approximately 0.5 cm. by 4.0 cm. was cut from each stomach adjacent and cranial to the tissue obtained

for mycologic examination. These sections were immediately placed in a 10% formalin solution. They were trimmed two to three days later and then embedded, sectioned, and stained. Hematoxylin and eosin and Grocott stains were obtained on each stomach for histopathologic examination.

The Grocott stained sections were examined for the presence of hyphae, pseudohyphae, or yeast cells. The hematoxylin and eosin sections were examined carefully and put into the classes defined by Muggenburg (1964).

RESULTS

The recovery of C. albicans from fecal samples for the first five weeks of the experiment was extremely low, only two recoveries being made from Lots M and S. Recovery from the three lots for the next six weeks is shown in Table 3.

Table 3—Recovery of C. albicans from Fecal Samples

Day	Lot C	Lot S	Lot M
35	6*	9	5
42	9	10	8
49	7	10	10
56	1	2	3
63	0	3	0
70	2	2	0

*The figures represent the number of pigs from which C. albicans was recovered, regardless of number of organisms.

Application of a Row X Column Chi^2 test to the weekly results from Table indicates that the results are independent of the treatment, i.e., the difference in dietary sugar (Table 4).

Table 4—Row X Column Chi^2 Test Applied to Results in Table 3

Day	Calculated Chi^2	Conclusion	Day	Calculated Chi^2	Conclusion
35	3.90	Accept*	56	1.25	Accept
42	2.22	Accept	63	6.67	Accept
49	6.67	Accept	70	2.31	Accept

*Independence rejected if calculated Chi^2 exceeds tabular Chi^2 of 7.38 at 0.025 with 2 degrees of freedom (Snedecor, 1956). If a tabular Chi^2 had been drawn from the 0.050 level, dependence would have been indicated for the samples from the 49th and 63rd days.

The samples from days 35, 42, and 49 were taken while the pigs were receiving continuous dosage of _C. albicans_ in their drinking water. These three samples from each lot were considered to be multiple samples from the same population for the purpose of applying a simple analysis of variance (Table 5).

Table 5—Analysis of Variance on Results from Days 42, 49, and 56

Source	Degrees of freedom	Sum of squares	Mean square	F
Lots	2	9.6	4.8	1.6
Within	6	18.0	3.0	

The same analysis was applied to the samples from days 56, 63, and 70, since these samples were collected while the pigs

were receiving few or no C. albicans (Table 6).

Table 6—Analysis of Variance on Results from Days 56, 63, and 70

Source	Degrees of freedom	Sum of squares	Mean square	F
Lots	2	2.55	1.28	0.80
Within	6	9.67	1.61	

In each of the above cases, a value of F, 5% with two and six degrees of freedom, exceeding 5.14 was necessary to reject the null hypothesis that the means of the three lots were the same.

No C. albicans was recovered from the sections of the gastric wall taken at slaughter. No fungal or yeast elements were observed on the Grocott-stained sections of the stomachs. C. albicans was recovered from the stomach contents swabs of 2 pigs in Lot M. A mucosal swab from one of these pigs also yielded C. albicans.

The gross and microscopic pathologic changes in the stomachs are recorded by lot in Table 7.

Table 7—Pathologic Changes in Esophageal Area of Stomachs

			Lot C*	Lot S	Lot M
Gross	Degree of keratinization[1]	K_0	1	3	0
		K_1	8	6	10
		K_2	0	1	0
	Other changes[2]	Normal	4	6	4
		Slight erosion	1	2	3
		Marked erosion	1	0	1
		Slight ulceration	2	1	2
		Marked ulceration	1	1	0
Microscopic[3]	Normal		2	4	3
	Epithelial change		3	2	0
	Acute erosion		0	1	3
	Subacute ulcer		3	2	4
	Chronic ulcer		1	1	0
	Scar		0	0	0

*One stomach in Lot C was not recovered.

[1]As defined by Bixby (1964).

[2]A subjective evaluation. Erosions are interpreted as involving only the epithelium. Ulcers also involve the deeper layers.

[3]As defined by Muggenburg (1964).

The average number of C. albicans recoveries/pig from the fecal samples as related to degree of microscopic pathologic change in the esophageal area of the stomach are shown (Table 8).

Table 8—Average Number of Recoveries of *C. albicans* Related to Pathologic Change

Pathologic change	Number of pigs	Average No. of *C. albicans* recoveries
Normal	9	3.1
Epithelial change	5	4.0
Acute erosion	4	2.5
Subacute ulcer	9	3.0
Chronic ulcer	2	2.5

Selected lot blood glucose averages are shown in Table 9.

Table 9—Lot Blood Glucose Averages

	Lot C	Lot S	Lot M
The 2 weeks prior to and the start of the experiment	53.2	54.6	51.3
The last 4 weeks of the experiment	57.1	58.8	55.5
Increase	3.9	4.2	4.2

The relation of blood glucose to number of *C. albicans* recoveries from fecal samples is shown in Table 10.

Table 10—Average Blood Glucose/Number of *C. albicans* Recoveries

Number of recoveries	Number of pigs	Blood glucose average*
1	3	56.8
2	5	57.2
3	13	55.7
4	8	57.5
7	1	55.0

*For entire experimental period.

The degree of pathologic change in the esophageal area of the stomach as related to blood glucose is shown in Table 11.

Table 11—Blood Glucose and Microscopic Pathologic Change

Degree of change	Number of pigs	Blood glucose average*
Normal	9	56.7
Epithelial change	5	56.6
Acute erosion	4	59.2
Subacute ulcer	9	55.3
Chronic ulcer	2	55.4

*For entire experimental period.

The litter effect on microscopic pathologic change and blood glucose average is shown in Table 12.

Table 12—Pathologic Change and Blood Glucose Average by Litter

	Litter 1	Litter 2	Litter 3	Litter 4
Normal	0	1	4	4
Epithelial change	0	2	2	1
Acute erosion	0	0	2	2
Subacute ulcer	4	2	1	2
Chronic ulcer	2	0	0	0
Blood glucose average for entire experiment	55.4	55.3	57.9	56.9

The microscopic pathologic changes and average blood glucose levels by sex are shown in Table 13.

Table 13—Pathologic Change and Blood Glucose Average of Barrows and Gilts

	Normal	Epithelial change	Acute erosion	Subacute ulcer	Chronic ulcer	Blood glucose for entire experiment
Barrows	4	4	0	7	1	56.2
Gilts	5	1	4	2	1	56.9

DISCUSSION

The diet of the control lot (C) was designed to be an average supplemented growing ration. The sugars were added to the diets of the experimental lots (S,M) to determine if a difference would be observed in growth of C. albicans and presence of pathologic changes in the stomachs.

Maltose and sucrose were chosen as the added sugars because both are readily utilized by C. albicans. Sucrose is sometimes used in artificial diets for younger pigs. Maltose would be present in small amount after mastication of feed due to the action of salivary amylase on starch (Ellenberger, 1887). It was theorized that increased porosity of feed, as in some high-heat treated pellets, might allow saliva to penetrate the feed, allowing the salivary amylase to act upon starch for a considerable time before being inactivated by stomach acid. Enough of the starch substrate in very finely ground feeds might be immediately available for hydrolysis by salivary amylase to result in an increased amount of sugar in the stomach. The resultant sugar would be maltose primarily, but D glucose and D glucose polysaccharides of varying lengths also would be present.

A comparison of the number of recoveries of C. albicans from the three lots indicated that the additional dietary sugar had little or no effect on the growth of the organism in the digestive tract. This is statistically supported on individual samples (Table 4) and multiple samples (Tables 5 and 6).

Failure to culture C. albicans from the stomach tissues obtained at slaughter was somewhat surprising. Bixby (1964) isolated the organism from 2 of 50 randomly selected pig stomachs in a survey. Griffing (1963) isolated C. albicans from 50 of 226 pig stomachs. However, only 100 of the 226 could be considered as survey stomachs, and none of these yielded the organism. The other 126 pigs were from nutrition trials, the Kansas Boar

Improvement Station, and other sources. None of the above pigs reported by Bixby and Griffing was purposely fed large numbers of C. albicans, as were the pigs in the present experiment.

The negative Grocott-stained sections of the esophageal area of the stomachs substantiated the negative stomach tissue culture results found in this experiment.

Degree of keratinization was quite consistent between the three lots (Table 7). Only four stomachs appeared completely normal. Twenty-four showed very slight keratinization (K_1). Some of these showed a slight yellow discoloration. Only one stomach showed slight keratinization (K_2) as evidenced by a slight roughened surface proliferation and a yellow color. Fewer severely keratinized stomachs were observed in this experiment than in those reported by Bixby (1964) and Griffing (1963).

Gross and microscopic pathologic changes correlated well within each lot. Very little difference in gross pathologic change was noted between the lots (Table 7). Lot M had seven of ten stomachs microscopically classified at least as severe as acute erosion, whereas Lot C had only four of nine and Lot S four of ten. However, Lots C and S each had one stomach classified as chronic ulcer compared to none for Lot M. If any difference was present, it would be a slightly more severe involvement of the stomachs of Lot M. A numerical scale could be assigned to the degrees of pathologic change to statistically compare the lots. However, this might be deceptive because the numerical scale would be arbitrarily assigned.

There is no correlation between *C. albicans* recoveries from fecal samples and degree of pathologic change present (Table 8). The pig which yielded the organism from its stomach mucosal and stomach contents swabs showed slight denudation grossly and subacute ulceration microscopically. The pig yielding *C. albicans* only from the stomach contents swab showed slight ulceration grossly and subacute ulceration microscopically. Each of these pigs yielded the organism three times from the fecal samples.

There is little difference between lots in blood glucose averages, both prior to and after being placed on different diets (Table 9). The number of *C. albicans* recoveries was not affected by blood glucose level (Table 10), and the degree of microscopic pathologic change was not affected by blood glucose level (Table 11).

The method for determining blood glucose was a screening type clinical test but would have shown marked differences.

The most striking comparison in the experiment was the litter difference in microscopic pathologic change (Table 12). Litters 2, 3, and 4 were very comparable, but litter 1 showed very severe involvement. All 6 littermates showed ulceration, compared to only 5 of 23 pigs showing ulceration in the other three litters. The sow of litter 1 had mastitis for several weeks, but the pigs exhibited no outward effects. However, the mastitic milk may have contributed to the increased ulceration in that litter. A genetic predisposition to ulceration should also be considered in this case.

Eight of 16 barrows showed stomach ulceration microscopically, while only 3 of 13 gilts showed ulceration (Table 13). Curtin (1963) reported a 23.57% incidence of ulceration in barrows compared to 17.82% in gilts.

CONCLUSIONS

Low levels of dietary sucrose and maltose did not affect growth of _C_. _albicans_ in the porcine digestive tract and did not affect the development of pathologic changes in the esophageal area of the stomach of the pigs in this experiment. Growth of _C_. _albicans_ had no effect on esophagogastric pathologic changes.

Blood glucose levels were not affected by dietary sugar. Blood glucose levels had no effect on growth of _C_. _albicans_ or esophagogastric pathologic changes in this experiment.

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REFERENCES

Allcroft, R., Burns, K. N., and Lewis, G.: Effect of High Levels of Copper in Rations for Pigs. Vet. Rec., 73 (July, 1961): 714-718.

Arkhipovets, A. I.: The Amylolytic Activity of Pigs' Saliva and Its Role in the Digestive Process. Vestnik Sel'skokhoz. Nauki, 7 (1962): 86-88. Original not seen. As in Biol. Abstr., 41 (Feb., 1963): 727.

Baker, E. D., and Cadman, L. P.: Candidiasis in Pigs in Northwestern Wisconsin. J.A.V.M.A., 142 (Apr., 1963): 763-767.

Barron, N. S., and O'Brien, J. D. P.: Letters to the Editor. Vet. Rec., 75 (Jan., 1963): 100-102.

Bixby, H. R.: The Effects of Candida albicans on the Esophagogastric Area of the Porcine Stomach. Kansas State University, M. S. Thesis (1964).

Blaxland, J. D., and Markson, L. M.: Observations on the Transmissability and Pathogenesis of Moniliasis in Turkey Poults. Brit. Vet. J., 110 (Apr., 1954): 139-145.

Bullard, J. F.: Gastric Ulcer in a Large Boar. J.A.V.M.A., 119 (Aug., 1951): 129.

Curtin, T. M., Goetsch, G. D., and Hollandbeck, R.: Clinical and Pathologic Characterization of Esophageal Ulcers in Swine. J.A.V.M.A., 143 (Oct., 1963): 854-860.

Dodd, D. C.: Hyostrongylosis and Gastric Ulceration in the Pig. New Zealand Vet. J., 8 (1960): 100-103.

Ellenberger, W., Arch. wiss. u. prakt. Tierheilk., 13 (1887): 188. Original not seen. As cited in Hudman, et al., J. Agric. and Food Chem., 5 (Sept., 1957): 691-693.

Gitter, M., and Austwick, P. K. C.: Mucormycosis and Moniliasis in a Litter of Suckling Pigs. Vet. Rec., 71 (Jan., 1959): 6-11.

Griffing, J. G.: A Study of the Etiology and Pathology of Gastric Ulcers in Swine. Kansas State University, Ph. D. Dissertation (1963).

Hudman, D. B., Friend, D. W., Hartman, P. A., Ashton, G. C., and Catron, D. V.: Digestive Enzymes of the Baby Pig. Pancreatic and Salivary Amylase. J. Agric. and Food Chem., 5 (Sept., 1957): 691-693.

Kapica, L., and Blank, F.: Growth of Candida albicans on Keratin as Sole Source of Nitrogen. Dermatologica, 115 (1957): 81-105.

Kernkamp, H. C. H.: Gastric Ulcers in Swine. Am. J. Path., 21 (1945): 111-113.

Kowalczyk, T., Hoekstra, W. G., Puestow, K. L., Smith, I. D., and Grummer, R. H.: Stomach Ulcers in Swine. J.A.V.M.A., 137 (Sept., 1960): 339-344.

Mahan, D. C., Pickett, R. A., Perry, T. W., Curtin, T. M., Beeson, W. M., and Featherston, W. R.: Influence of Ration Particle Size on the Incidence of Ulcers in Swine. J. Anim. Sci., 23 (Aug., 1964): 883.

Muggenburg, B. A., McNutt, S. H., and Kowalczyk, T.: Pathology of Gastric Ulcers in Swine. Am. J. Vet. Res., 25 (Sept., 1964): 1354-1365.

Nickerson, W. J., and Mankowski, Z.: A Polysaccharide Medium of Known Composition Favoring Chlamydospore Formation in Candida albicans. J. Inf. Dis., 92 (Jan.-Feb., 1953): 20-25.

Nuwer, A. J., Perry, T. W., Pickett, R. A., Curtin, T. M., and Beeson, W. M.: Value of Various Additives to Ulcer-Producing Gelatinized Corn Diets Fed to Swine. J. Anim. Sci., 22 (Nov., 1963): 1129.

Nuwer, A. J., Perry, T. W., Pickett, R. A., Curtin, T. M., Featherston, W. R., and Beeson, W. M.: Value of Various Additives to Ulcer-Producing Gelatinized Corn Diets Fed to Swine. J. Anim. Sci., 24 (Feb., 1965): 113-119.

Osborne, A. D., McCrea, M. R., and Manners, M. J.: Moniliasis in Artificially Reared Pigs and Its Treatment with Nystatin. Vet. Rec., 72 (Mar., 1960): 237-241.

Pagano, J., Levin, J. D., and Trejo, W.: Diagnostic Medium for Differentiation of Species of Candida. Antibiotics Annual (1957-1958): 137-143.

Perry, T. W., Shively, J. E., Jiminez, A. A., Pickett, R. A., Curtin, T. M., and Beeson, W. M.: Effect of Type of Ration on the Incidence of Swine Gastric Ulcers. J. Anim. Sci., 21 (1962): 1008-1009.

Perry, T. W., Jiminez, A. A., Shively, J. R., Curtin, T. M., Pickett, R. A., and Beeson, W. M.: Incidence of Gastric Ulcers in Swine. Science, 139 (Jan., 1963): 349-350.

Reese, N. A., Muggenburg, B. A., Kowalczyk, T., Hoekstra, W. G., and Grummer, R. H.: Factors Influencing Gastric Ulcers in Swine. J. Anim. Sci., 22 (Nov., 1963): 1129.

Reese, N. A., Muggenburg, B. A., Kowalczyk, T., Hoekstra, W. G., and Grummer, R. H.: The Influence of Corn, Wheat, and Oats on Gastric Ulcers in Swine. J. Anim. Sci., 23 (Aug., 1964): 890.

Rothenbacker, H., Nelson, L. W., and Ellis, J. D.: The Stomach Ulcer-Gastrorrhagia Syndrome in Michigan Pigs. Vet. Med., 58 (Oct., 1963): 806-816.

Snedecor, G. W.: Statistical Methods. 5th Ed., Iowa State University Press, Ames, Iowa, 1956.

RELATIONSHIPS BETWEEN DIETARY SUGAR, CANDIDA ALBICANS, AND GASTRIC ULCERS IN SWINE

by

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AN ABSTRACT OF A MASTER'S THESIS

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Thirty 19-week-old pigs from four litters were randomly assigned to three lots. One lot was fed a normal growing ration. The second lot was fed the growing ration plus 2.0% sucrose. The third was fed the growing ration plus 2.0% maltose. All pigs were fed viable *Candida albicans* the first seven weeks of the experiment but not the last five weeks.

Fecal samples taken weekly were cultured for *C. albicans*. Blood glucose determinations were performed on blood samples each week. At slaughter, stomachs were collected for mycologic, gross pathologic, and histopathologic examination of the glandless esophageal area.

No significant difference between lots was present in number of recoveries of *C. albicans* from fecal samples during or after feeding of the organisms, or in the presence of gross pathologic or histopathologic change. No association was detected between number of recoveries of *C. albicans* and pathologic change. Seven stomachs exhibited ulceration grossly compared to eleven microscopically. Blood glucose level had no effect on either the number of *C. albicans* recoveries or pathologic change.

The most striking comparison obtained was a litter difference. Six of six pigs from one litter exhibited ulceration microscopically compared to 5 of 23 from the other three litters (one stomach was not recovered). The sow of the litter with high ulcer incidence had mastitis for several weeks, although there was no change evident in her nursing pigs.